Nasal gene expression shows a distinct signature in type 2–high asthma but not in type 2–low disease

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Background: Type 2 (T2)-low asthma is defined by low levels of T2 inflammation and is associated with resistance to inhaled corticosteroids. Its molecular mechanisms are mostly unknown, and treatment options are limited. We previously showed that nasal brush transcriptomes differ between asthma and controls, reflecting disease-relevant biology.

Objective: We explored nasal gene expression related to T2-low asthma in the ATLANTIS cohort.

Methods: We compared nasal brush RNA sequencing data between 82 T2-low and 63 T2-high asthma patients and 57 controls. T2-low asthma was defined as blood eosinophil counts < 0.15 × 10^9/L and FENO < 25 ppb and T2-high as blood eosinophil counts > 0.3 × 10^9/L and FENO > 25 ppb. Weighted gene coexpression network analysis (WGCNA) was applied to identify gene modules associated with T2-low asthma. The BAMSE and U-BIOPRED cohorts were used for replication analyses.

Results: Although differentially expressed genes were found in patients with T2-high asthma across all 3 cohorts, no differentially expressed genes were observed in individuals with T2-low disease compared to controls in ATLANTIS, nor consistently across other cohorts. Our assessment of molecular heterogeneity could not attribute this result to greater intersample variability within the T2-low group. WGCNA in ATLANTIS identified "black" and "purple" gene modules linked to T2-low asthma, with genes enriched in T-cell immunity and ribosomal RNA biology pathways, respectively. The "black" module was replicated in U-BIOPRED and showed the same direction in BAMSE.

Conclusion: T2-high asthma shows a distinct nasal gene expression signature compared to healthy controls, while patients with T2-low asthma exhibit no consistent changes. Future studies should explore T-cell immunity in T2-low asthma and integrate lower airway multiomics data. (J Allergy Clin Immunol 2026;157:879-89.)

Key words: *Asthma, endotype, nasal, gene expression, type2-low asthma*

Asthma is a chronic inflammatory airway disease characterized by variable airflow obstruction in association with symptoms of wheezing and dyspnea. During the last decade, asthma phenotyping has advanced individualized disease management. For patients with severe type 2 (T2)-high asthma, characterized either by high blood eosinophil counts or exhaled nitric oxide levels, treatment has significantly improved with the introduction of anti–IL-5/IL-5R and anti–IL-4R antibodies.[1,2] In contrast to the T2-high asthma phenotype, few advancements have been made for the treatment of T2-low asthma, and the underlying molecular mechanisms of this phenotype remain less well understood.[3,4]

RNA sequencing of nasal brushes has been suggested to be useful as a tool to identify asthma-specific and biologically relevant gene expression changes with alterations occurring in the upper airways, reflecting those that occur in the lower airways.[5-9] On the basis of this concept, known as *united airways,* we

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Abbreviations used

ATLANTIS: Assessment of Small Airways Involvement in Asthma Study
BAMSE: Barn [Child], Allergy, Milieu, Stockholm, Epidemiological study
DEG: Differentially expressed gene
DGE: Differential gene expression
FDR: False discovery rate
Feno: Fractional exhaled nitric oxide
GO: Gene Ontology (geneontology.org)
GSEA: Gene set enrichment analysis
ICS: Inhaled corticosteroids
MHC: Major histocompatibility complex
T2: Type 2
U-BIOPRED: Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes study
WGCNA: Weighted gene correlation network analysis

previously identified an asthma-specific nasal gene expression profile.[10] Moreover, we showed that a cluster analysis that is solely based on the nasal expression of asthma-associated genes reveals clinically relevant distinct asthma endotypes.[10]

The aim of the current study was to extend our earlier findings to obtain novel insights into the underlying biology of T2-low asthma.[5,11,12] To this end, we used available clinical and nasal RNA sequencing data from the Assessment of Small Airways Involvement in Asthma (ATLANTIS) study with replication in Barn [Child], Allergy, Milieu, Stockholm, Epidemiological (BAMSE) study and the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) study cohorts and compared gene expression levels between T2-high and T2-low asthma patients on the one hand to those of healthy controls on the other hand. A distinct signature for T2-high asthma was detected, but not for T2-low asthma, across all 3 cohorts. A gene module associated with T-cell immunity and major histocompatibility complex (MHC) was found in T2-low asthma in ATLANTIS and replicated in U-BIOPRED.

METHODS

Study design, definition, and RNA sequencing

The ATLANTIS study was a multinational prospective observational study that included both asthma patients and nonasthmatic controls aged 18 to 65 years. Asthma diagnosis was confirmed by a chest physician in accordance with the 2012 Global Initiative for Asthma severity staging. Detailed study design, inclusion and exclusion criteria, and main baseline clinical findings have been described previously.[13] Nasal brushes of the inferior turbinate were collected in a subset of centers in Europe, the United States, and Canada at the baseline visit. ATLANTIS study participants were followed up for 1 year. A description of RNA sequencing sample preparation, quality control, and processing has been previously published.[10] The medical ethics committee of each participating center approved the study protocol, and all patients provided written informed consent. Sex was self-reported.

T2-low asthma was defined by blood eosinophil counts $< 0.15 \times 10^9$ cells/L and, when measured, FENO < 25 ppb.[14-17] T2-high asthma was defined by blood eosinophil counts $> 0.3 \times 10^9$ cells/L and, when measured, FENO > 25 ppb. Disease that did not fulfill the criteria for T2-low or -high asthma was labeled as undetermined. T2-low and T2-high stable asthma was defined as having the abovementioned criteria at each of the 3 visits. Participants who received therapy with systemic corticosteroids or biologics were excluded from the analysis.

A T2-low sputum paucigranulocytic phenotype was defined as a T2-low phenotype with sputum eosinophils < 2% and sputum neutrophils < 50% (n = 18); T2-low sputum neutrophilic phenotype was defined as T2-low phenotypes with sputum eosinophils < 2% and sputum neutrophils > 50% (n = 21), as described previously.[18]

Statistical analysis

Comparison of baseline clinical characteristics between T2-low and -high asthma was performed by the R software package 'tableone' v0.13.2. This package uses a *t* test for normally distributed variables, a Mann-Whitney *U* test for nonnormally distributed variables, and a chi-square test for categorical variables.

Differential gene expression (DGE) analysis was conducted by the 'edgeR' v4.2.0 package, including the 'calcNormFactors' function with a trimmed mean of *M* values to normalize for effective library sizes. We assessed the effect of the sample collection hospital on the nasal gene expression by drawing 95% confidence intervals around each group on the principal component analysis plots (PC1-PC5). Because none of the groups was seen as an outlier, we concluded that adjustment for hospital was not necessary (see Fig E1 in the Online Repository available at www.jacionline.org). A negative binomial generalized linear model was constructed using all the samples (nonasthmatic, T2-low, T2-high, and undetermined subgroup) adjusting for sex, age, and smoking status (factor with 3 levels: current, ex-, and never smokers) to identify differentially expressed genes (DEGs) between T2-low asthma and healthy controls on the one hand and T2-high asthma and healthy controls on the other hand.

One possible explanation for the lack of identified DEGs in T2-low asthma is the heterogeneity of this phenotype. To assess intersample gene expression dissimilarity within each group (T2-high, T2-low asthma, and healthy), we calculated pairwise Euclidean distances based on normalized expression values (CPMs). To this end, we used the 'dist' function after filtering out low-expressed genes with the 'filterByExpr' function. Euclidean distances between the groups were compared by the Mann-Whitney *U* test. In addition, we performed principal component analysis and clustering analysis with gap statistics to further heterogeneity, as described in more detail in the Methods section in this article's Online Repository available at www.jacionline.org.

Gene set enrichment analysis (GSEA) was conducted in the 'clusterProfiler' and ReactomePA packages using the Gene Ontology (GO; geneontology.org) and Reactome databases, respectively, to identify pathways enriched in the resulting gene list from the DGE analysis between T2-low asthma and nonasthmatic controls. Genes were ranked by $(-\log_{10}P\text{value})*\pm 1$ (depending on the direction of the log fold change).

Cell type deconvolution

We performed cell type deconvolution analysis to assess differences in the cell composition in T2-high and T2-low asthma compared to healthy controls. The CIBERSORTx algorithm[19] was used to estimate cell proportions in nasal brush bulk RNA

samples using a cell-type signature matrix derived from single-cell nasal brush data from the Human Lung Cell Atlas.[20] The following cell types were present in the signature matrix: basal resting, club, dendritic, goblet, hillock, multiciliated lineage, and T-cell lineage. Bulk gene expression data was normalized with the counts-per-million (CPM) method, and marker genes between signature and deconvolved matrices were matched by Ensembl gene IDs. Only one subject had a nonzero proportion of T-cell lineage, so this cell type was not tested for the cell type differences between the groups. The comparison was performed by the Mann-Whitney *U* test. The false discovery rate (FDR; Benjamini-Hochberg procedure) was used for multiple testing correction.

Weighted gene correlation network analysis

We used weighted gene correlation network analysis via the WGCNA R package to construct a gene coexpression network to further explore gene expression changes in the nasal epithelium in T2-low asthma.[21] The network was built using gene expression data from nonasthmatic controls and T2-low asthma subjects. Genes with fewer than 15 counts in more than 75% of the samples were excluded, and variance stabilizing transformation was applied to the remaining gene counts. The power parameter $\beta = 7$ (soft threshold) was used on the basis of the scale-free topology criterion. Signed type of adjacency matrix was then transformed into a topological overlap matrix, and genes were hierarchically clustered on the basis of matrix similarity. Coexpression gene modules were identified using the dynamic tree cut algorithm with a dendrogram cut height set at 0.25. The gene modules are named using the default naming conventions from the WGCNA package. The module eigengene was defined as the first principal component of the module. The module membership of the genes in each gene module was calculated as the correlation of the eigengene and the normalized gene expression value.

To evaluate the relationship between the identified modules and T2-low asthma, a *t* test was performed on the module eigengene between controls and T2-low asthma subjects. FDR was used for multiple testing correction. GO overrepresentation analysis using genes from the modules associated with T2-low asthma was performed by the 'enrichGO' function from 'clusterProfiler' v4.12.0 package. Genes used as input for the WGCNA served as a background list. FDR was used for multiple testing correction; pathways with FDR < .05 were considered significantly enriched.

Replication in BAMSE and U-BIOPRED cohorts

Clinical characteristics and nasal gene expression data from the BAMSE study at 24 years' follow-up were used for this replication study.[22-25] In the BAMSE cohort, asthma was defined as either ever doctor's diagnosis of asthma together with symptoms of breathing difficulties in the last 12 months before the date of the questionnaire, or the receipt of asthma medication occasionally or regularly in the last 12 months. Detailed descriptions of the BAMSE cohort and RNA sequencing processing steps are described in the Methods section in the Online Repository. T2-high and T2-low asthma were defined the same way as in ATLANTIS, with the undetermined group included with the remaining asthma patients (n = 24). Because of the small size of the T2-high group in the BAMSE cohort (n = 6), it was combined with the undetermined group for the replication analysis, so from here on out, we refer to it as T2-high asthma. In total, we analyzed 336 samples from the BAMSE cohort: T2-high (n = 30), T2-low (n = 24), and controls (n = 282). DGE analysis was performed in the BAMSE cohort by contrasting T2-high asthma and T2-low asthma versus controls as in ATLANTIS, adjusting for age, sex, smoking status, RNA integrity number, and plate.

Microarray gene expression data from the nasal brush and clinical characteristics from the U-BIOPRED were used as a second independent cohort for the replication in this study. Detailed descriptions of the U-BIOPRED cohort have been published previously.[26] Nasal brush microarray processing is described in the Methods section in the Online Repository. T2-high and T2-low asthma were defined the same way as in ATLANTIS. DGE analysis was performed by the 'limma' package by applying linear modeling and empirical Bayes moderation by contrasting T2-high asthma (n = 9) and T2-low asthma (n = 16) versus controls (n = 23) and adjusting for age, sex, and smoking status (current, ex-, and never smoker).

Identified T2-high genes in ATLANTIS cohort were used as two gene sets in 2-tailed GSEA where genes identified in T2-high asthma in BAMSE and U-BIOPRED were ranked by $(-\log_{10}P\text{value})*\pm 1$ (depending on the direction of change) and used as a background list. The GSEA analysis was performed by the 'fgsea' package.[27]

RESULTS

Baseline characteristics of T2-high and T2-low asthma patients

A total of 82 patients had T2-low and 63 T2-high asthma in the ATLANTIS study. We compared their clinical characteristics with the 57 healthy controls (Table I). Patients with T2-low asthma were more often female (75.6%) than controls (52.6%), which was not the case for T2-high asthma (54%). There was no difference in body mass index between T2-low and T2-high asthma patients and controls (T2-low, 25.8 kg/m^2; T2-high, 26.1 kg/m^2; nonasthmatic controls, 26.0 kg/m^2). Both asthma phenotypes had a higher percentage of individuals with IgE sensitization, as indicated by positive Phadiatop test result (T2-low, 72%; T2-high, 83%) compared to controls (42%). Both T2-low and T2-high asthma patients had small airway dysfunction, as reflected by a lower forced expiratory flow at 50% of forced vital capacity (T2-low, 77.4; T2-high, 61.5) and higher residual volume/total lung capacity (T2-low, 97.6; T2-high, 110.6).

Twenty-two patients had stable T2-low (36.7% of those who had T2-low at the first visit) and 31 (54.4%) had stable T2-high asthma across all 3 visits (Fig 1, *A*). Participants' baseline clinical characteristics are presented in Table II.

T2-low asthma showed no genome-wide DEGs compared to healthy controls

We identified 371 higher and 192 lower expressed genes in T2-high asthma compared to nonasthmatic controls (FDR < .05; Fig 1, *B,* and see Table E1 in the Online Repository available at www.jacionline.org). We did not observe any significant DEGs when we compared T2-low asthma with nonasthmatic controls. Similarly, patients with T2-low stable asthma did not show any genome-wide DEGs compared to nonasthmatic controls, while T2-high stable asthma patients showed 35 higher and 46 lower expressed genes (see Table E2 in the Online Repository). When the disease of T2-low asthma patients was subdivided into sputum neutrophilic

TABLE I. Baseline clinical characteristics of participants

Characteristic	Healthy	T2-low		T2-high		*P* (high vs low)
		Value	*P*	Value	*P*	
No. of participants	57	82		63		
Male, no. (%)	27 (47.4)	20 (24.4)	.008	29 (46.0)	1	.011
Age (years), mean (SD)	46 (13)	43 (14)	.322	43 (14)	.289	.892
Smoking status			.117		.567	.379
Current smoker, no. (%)	0	5 (6.1)		1 (1.6)		
Ex-smoker, no. (%)	10 (17.5)	18 (22.0)		13 (20.6)		
Nonsmoker, no. (%)	47 (82.5)	59 (72.0)		49 (77.8)		
Body mass index (kg/m^2), mean (SD)	26.0 (4.1)	25.8 (5.2)	.755	26.1 (5.3)	.952	.724
Positive Phadiatop test result, n/N (%)	21/50 (42.0)	49/68 (72.1)	.002	45/54 (83.3)	<.001	.210
Disease duration (years), median [IQR]		16.37 [7.93, 25.70]		20.71 [10.09, 29.34]		.106
Age at diagnosis (years), median [IQR]		23.75 [10.04, 37.13]		20.86 [5.59, 35.48]		.117
ICS receipt, no. (%)		63 (76.8)		52 (82.5)		.526
Daily ICS dose (μg),† median [IQR]		800.00 [400.00, 1000.00]		800.00 [400.00, 1000.00]		.931
ACQ6 score, median [IQR]		0.83 [0.17, 1.50]		1.00 [0.50, 1.67]		.201
Blood eosinophils (10^9/L), median [IQR]	0.1 [0.1, 0.2]	0.1 [0.1, 0.1]	.001	0.5 [0.4, 0.6]	<.001	<.001
Blood neutrophils (10^9/L), mean (SD)	3.5 (1.4)	3.7 (1.4)	.410	4.0 (1.7)	.084	.245
Blood monocytes (10^9/L), mean (SD)	0.5 (0.6)	0.5 (0.1)	.789	0.6 (0.2)	<.001	<.001
Sputum eosinophils (% of nonsquamous cells), median [IQR]	0.1 [0.0, 0.3]	0.0 [0.0, 0.4]	.474	4.0 [0.9, 30.5]	<.001	<.001
Sputum neutrophils (% of nonsquamous cells), median [IQR]	51.3 [23.7, 65.7]	51.1 [35.5, 75.4]	.404	46.1 [25.9, 59.5]	.797	.278
FEV_1 (% predicted), mean (SD)	101.0 (11.64)	89.1 (13.8)	<.001	79.7 (17.8)	<.001	.001
FENO was measured, no. (%)	52 (91.2)	64 (78.0)		51 (81.0)		
FENO (ppb), median (IQR)	18.0 [11.8, 27.0]	16.0 [11.8, 20.0]	.045	44.0 [37.5, 71.5]	<.001	<.001
RV/TLC (% predicted), mean (SD)	89.1 (19.5)	97.6 (19.0)	.014	110.6 (27.7)	<.001	.001
R5-R20 (% predicted), median [IQR]	93.1 [0.0, 135.6]	219.1 [35.2, 429.5]	.004	297.9 [71.3, 493.2]	<.001	.369
S_{cond}*VT (% predicted), median [IQR]	58.6 [28.8, 86.4]	54.7 [38.4, 100]	.464	112.9 [54.5, 166.4]	.003	.012
S_{acin}*VT (% predicted), median [IQR]	110.5 [75.4, 139.3]	103.46 [80.3, 178.7]	.485	130.0 [93.3, 170.3]	.047	.321
FEF_{50} (% predicted), mean (SD)	99.8 (21.8)	77.4 (26.0)	<.001	61.5 (28.3)	<.001	.001
One or more exacerbation last year, no. (%)		11 (13.4)		6 (9.5)		.644
Systolic blood pressure (mmHg), mean (SD)	123.40 (13.30)	122.90 (14.73)	.838	122.56 (15.77)	.752	.892
Diastolic blood pressure (mmHg), mean (SD)	79.28 (10.14)	76.55 (8.33)	.084	75.84 (11.51)	.086	.668

ACQ6, Asthma Control Questionnaire 6; *FEF_{50},* forced expiratory flow at 50% of predicted normal value; *FEV_1,* forced expiratory volume in 1 second; *IQR,* interquartile range; *R5-R20,* resistance at 5 Hz–resistance at 20 Hz; *RV/TLC,* residual volume/total lung capacity; *S_{acin}*VT,* ventilation homogeneity of acinar zone of lung; *S_{cond}*VT,* ventilation heterogeneity in conductive zone of lung; *SD,* standard deviation.

†Beclomethasone equivalent in patients receiving ICS or ICS and long-acting β_2-agonists. Mean and SD were calculated for normally distributed data; and median with quartile 1 and quartile 3 for nonnormally distributed data. Testing comprised *t* test for normally distributed variables, Mann-Whitney *U* test for nonnormally distributed variables, and chi-square test for categorical variables.

and sputum paucigranulocytic phenotypes and compared to the disease of nonasthmatic controls, no significant DEGs (FDR < .05) were observed. GSEA using genes ranked by differential statistics between T2-low asthma and nonasthmatic controls demonstrated enrichment of ribosomal and translation-related pathways (see Fig E2, Fig E3, Table E3, and Table E4 in the Online Repository).

Assessment of molecular heterogeneity within T2-high, T2-low asthma, and healthy control groups

The pairwise Euclidian distances among patients with T2-high asthma were significantly higher than those of patients with T2-low asthma and healthy controls (Fig 2, *A*, $P = 6.71 \times 10^{-71}$, $P = 9.99 \times 10^{-61}$). In addition, because heterogeneity estimates are not typically included in conventional gene expression analyses, we also used alternative approaches such as principal component analysis and clustering analysis. These methods produced results consistent with the observations mentioned above (see Fig E4 in the Online Repository available at www.jacionline.org).

Cell type proportions and gene modules associated with T2-low asthma

We estimated cell type proportions in nasal brush samples to investigate differences in cell type composition in T2-low and T2-high asthma patients. Club cell proportions were significantly higher among T2-low asthma subjects compared to nonasthmatic controls ($P_{adj} = .033$, Fig 2, *B*).

To investigate more global changes on the transcriptomic level in T2-low asthma in addition to gene-by-gene analysis, we performed weighted gene correlation network analysis (WGCNA). This method identified 12 coexpression gene modules. Of these, 3 modules were associated with T2-low asthma on nominal *P* value: "black," "greenyellow," and "purple" ($P = .024$, .01, and .01, respectively, Fig 2, *C*), which included 111, 27, and 88 genes, respectively (see Tables E5-E7 in the Online Repository available at www.jacionline.org). Overrepresentation analysis using the GO databases showed that the "black" module was associated with immune related pathways, specifically T-cell regulation and MHC protein complex. The genes with the top module membership

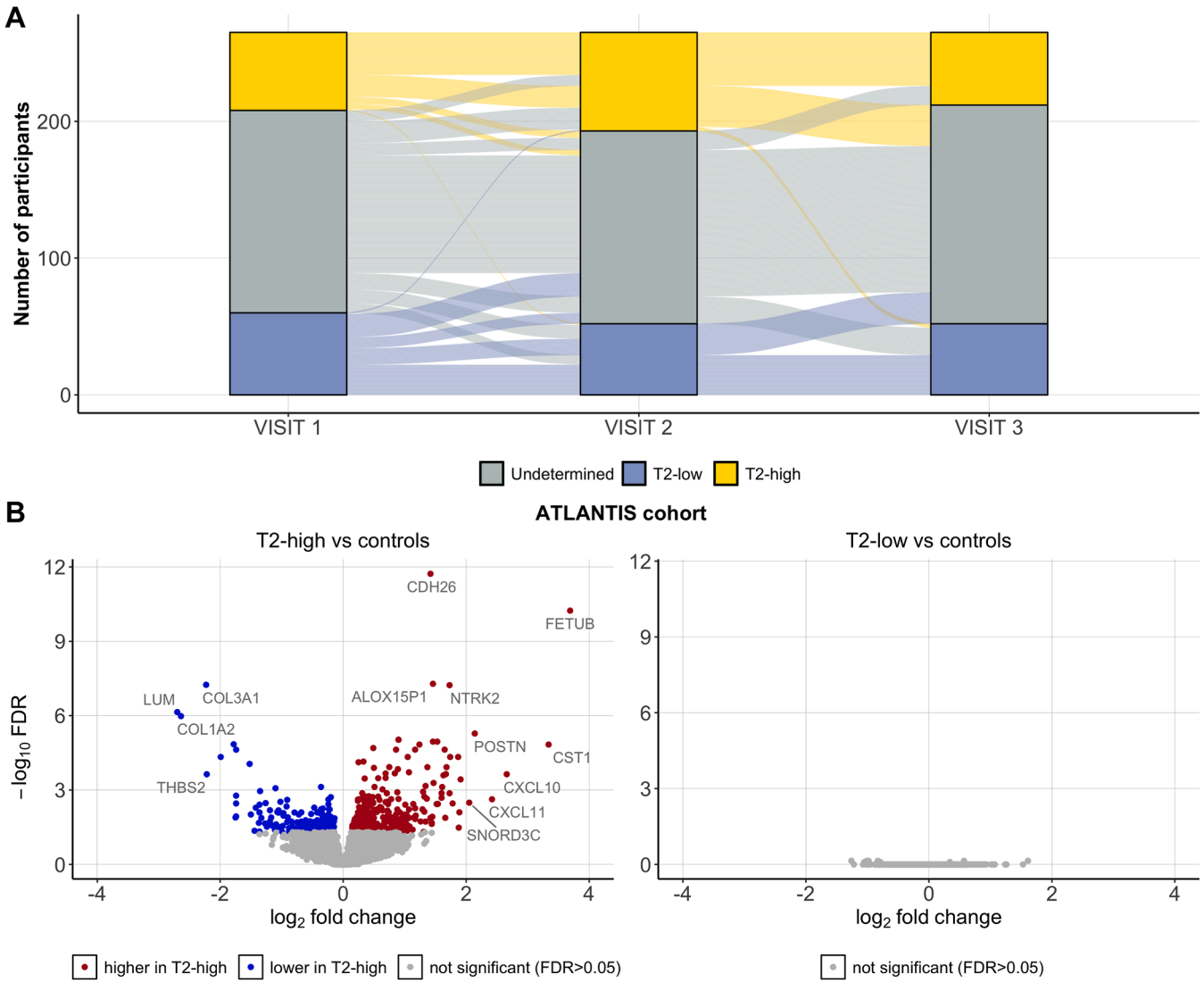


FIG 1. **(A)** Distribution of ATLANTIS study participants categorized into T2-low, T2-high, and undetermined asthma groups. Each *line* represents a single participant and tracks their asthma group status over 3 visits, which were conducted every 6 months. **(B)** Volcano plot representation of nasal brushes DGE analysis in ATLANTIS cohort. Significance level was defined as FDR < .05. Model was corrected for age, sex, and smoking status.

were HLA-DPA1 and HLA-DPB1. The "purple" module was related to ribosome biology and translation (see Figs E5-E8 and Tables E5, E6, E8, and E9 in the Online Repository). The "greenyellow" module included sex chromosome–encoded genes, such as *XIST, ZFX, ZFY,* and *JPX* (see Fig E9, Fig E10, Table E7, and Table E10 in the Online Repository). This module might reflect the higher proportion of female subjects, which we observed in patients with T2-low asthma. The "black" gene module showed significant correlation with predicted proportions of dendritic cells (Spearman correlation coefficient = 0.59, $P = 1.3 \times 10^{-14}$, see Fig E11 in the Online Repository) but no evidence of significant correlation to abundance of any other cell types tested. The "black" gene module was not significantly associated with any of the clinical variables (see Table E11 in the Online Repository).

Replication in two independent cohorts: BAMSE and U-BIOPRED

The baseline clinical characteristics of the participants in the BAMSE and U-BIOPRED cohorts are available in Tables E12 and E13 in the Online Repository available at www.jacionline.org. When comparing patients with T2-high asthma to healthy controls in the BAMSE and U-BIOPRED cohorts, we found a consistent signal, with many significantly DEGs common in the 3 cohorts (Fig 3, and see Tables E14 and E15 in the Online Repository).

The comparison between T2-low asthma patients and healthy controls in BAMSE and U-BIOPRED was also consistent with our results from the ATLANTIS cohort. In the BAMSE cohort, only the *CYP1A1* gene was identified as significantly more highly expressed in the T2-low patients compared to controls (Fig 3, *A,* and see Fig

TABLE II. Baseline clinical characteristics of patients with stable T2-high and T2-low asthma

Characteristic	Healthy	Stable T2-low		Stable T2-high		*P* stable (high vs low)
	Healthy	Value	*P*	Value	*P*	
No. of participants	57	22		31		
Male, no. (%)	27 (47.4)	6 (27.3)	.171	17 (54.8)	.655	.087
Age, years, mean (SD)	45.51 (13.41)	46.09 (11.68)	.858	43.29 (14.51)	.473	.457
Smoking status			.068		.379	.598
Current smoker, no. (%)	0	2 (9.1)		1 (3.2)		
Ex-smoker, no. (%)	10 (17.5)	3 (13.6)		6 (19.4)		
Nonsmoker, no. (%)	47 (82.5)	17 (77.3)		24 (77.4)		
body mass index (kg/m2) mean (SD)	26.03 (4.13)	26.96 (5.63)	.419	25.17 (4.89)	.390	.224
Positive Phadiatop test result, n/N (%)	21/50 (42.0)	12/16 (75.0)	.044	19/24 (79.2)	.006	1.000
Disease duration (years), median [IQR]		12.8 [7.4, 21.8]		19.2 [7.5, 23.7]		.829
Age at diagnosis (years), median [IQR]		32.9 [14.4, 36.9]		24.6 [10.8, 41.1]		.899
GINA, no. (%)						.365
1		3 (13.6)		6 (19.4)		
2		1 (4.5)		5 (16.1)		
3		7 (31.8)		5 (16.1)		
4		11 (50.0)		15 (48.4)		
ICS receipt, no. (%)		19 (86.4)		26 (83.9)		1.000
Daily ICS dose (μg),† median [IQR]		800.00 [500.00, 1,000.00]		800.00 [400.00, 800.00]		.224
ACQ6 score, median [IQR]		0.9 [0.3, 1.4]		0.8 [0.5, 1.3]		.906
Blood eosinophils (10^9/L), median [IQR]	0.1 [0.1, 0.2]	0.1 [0.1, 0.1]	.001	0.5 [0.4, 0.6]	<.001	<.001
Blood neutrophils, (10^9/L), mean (SD)	3.5 (1.4)	4.0 (1.6)	.204	4.2 (1.8)	.054	.658
Blood monocytes (10^9/L), mean (SD)	0.5 (0.2)	0.5 (0.2)	.702	0.6 (0.2)	<.001	.018
Sputum eosinophils (% of nonsquamous cells), median [IQR]	0.1 [0.0, 0.3]	0.0 [0.0, 0.1]	.267	3.0 [1.1, 25.6]	.001	.001
Sputum neutrophils (% of nonsquamous cells), median [IQR]	51.3 [23.7, 65.7]	54.60 [32.8, 75.3]	.537	47.3 [22.5, 53.3]	.442	.273
FEV_1 (% predicted), mean (SD)	101.0 (11.6)	88.0 (12.8)	<.001	82.5 (19.4)	<.001	.259
FENO (ppb), median (IQR)	18.0 [11.8, 27.0]	16.0 [14.0, 19.0]	.358	51.0 [42.5, 91.5]	<.001	<.001
RV/TLC (% predicted), mean (SD)	89.1 (19.5)	99.8 (22.2)	.044	111.6 (25.8)	<.001	.097
R5-R20 (% predicted), median [IQR]	93.1 [0.0, 135.6]	273.4 [105.93, 700.1]	.006	222.4 [44.2, 505.9]	.022	.509
S_{cond}*VT (% predicted), median [IQR]	58.6 [28.8, 86.4]	62.1 [32.0, 108.1]	.697	129.2 [67.8, 173.3]	.001	.031
S_{acin}*VT (% predicted), median [IQR]	110.5 [75.4, 139.3]	81.2 [66.5, 100.9]	.312	122.6 [93.3, 171.0]	.089	.060
FEF_{50} (% predicted), mean (SD)	99.7 (21.7)	74.5 (24.5)	<.001	64.3 (31.3)	<.001	.228
One or more exacerbation last year, no. (%)		1 (4.5)		4 (12.9)		.583
Systolic blood pressure (mmHg), mean (SD)	123.40 (13.30)	121.86 (12.40)	.640	122.32 (18.21)	.078	.919
Diastolic blood pressure (mmHg), mean (SD)	79.28 (10.14)	76.00 (8.69)	.185	75.13 (10.92)	.751	.757

ACQ6, Asthma Control Questionnaire 6; *FEF_{50},* forced expiratory flow at 50% of predicted normal value; *FEV_1,* forced expiratory volume in 1 second; *GINA,* Global Initiative for Asthma; *IQR,* interquartile range; *R5-R20,* resistance at 5 Hz–resistance at 20 Hz; *RV/TLC,* residual volume/total lung capacity; *S_{acin}*VT,* ventilation homogeneity of acinar zone of lung; *S_{cond}*VT,* ventilation heterogeneity in conductive zone of lung; *SD,* standard deviation.

†Beclomethasone equivalent in patients receiving ICS or ICS and long-acting β_2-agonists. Mean and SD were calculated for normally distributed data; and median with quartile 1 and quartile 3 for nonnormally distributed data. Testing comprised *t* test for normally distributed variables, Mann-Whitney *U* test for nonnormally distributed variables, and chi-square test for categorical variables.

E12 and Table E16 in the Online Repository available at www.jacionline.org; FDR = 9.41×10^{-8}, log fold change = 4.80). This result was potentially driven by a single subject with T2-low asthma with an extremely high expression of *CYP1A1*. After removing this subject and running the analysis again, we found that *CYP1A1* expression was not significantly different (FDR = .66). In the U-BIOPRED cohort, the *CHST2* gene was significantly more lowly expressed in the subjects with T2-low asthma compared to controls (Fig 3, *B,* and see Fig E13 and Table E17 in the Online Repository; FDR = .01, log fold change = −1.69). However, it was not different on the nominal *P* value level in either the ATLANTIS or BAMSE cohort (ATLANTIS *P* = .64, BAMSE *P* = .36).

The "black" gene module (as defined in WGCNA analysis of the ATLANTIS cohort) was significantly different in subjects with T2-low asthma compared to controls and in the same direction in the U-BIOPRED cohort as in the ATLANTIS cohort (see Fig E14 in the Online Repository available at www.jacionline.org; *P* = .0396). No significant difference was observed for the gene coexpression modules "black" and "purple" in the BAMSE cohort between healthy and patients with T2-low asthma; however, the direction of the differences was concordant with that observed in ATLANTIS (see Fig E15 in the Online Repository). There was no significant difference in cell type proportions between T2-low and healthy controls in BAMSE and U-BIOPRED cohorts (see Figs E16 and E17 in the Online Repository).

DISCUSSION

In this study, we found that patients with T2-high asthma exhibited a distinct nasal gene expression signature compared to healthy controls in robust findings that were replicated in two independent cohorts. Patients with T2-low asthma did not consistently show significant expression differences on an

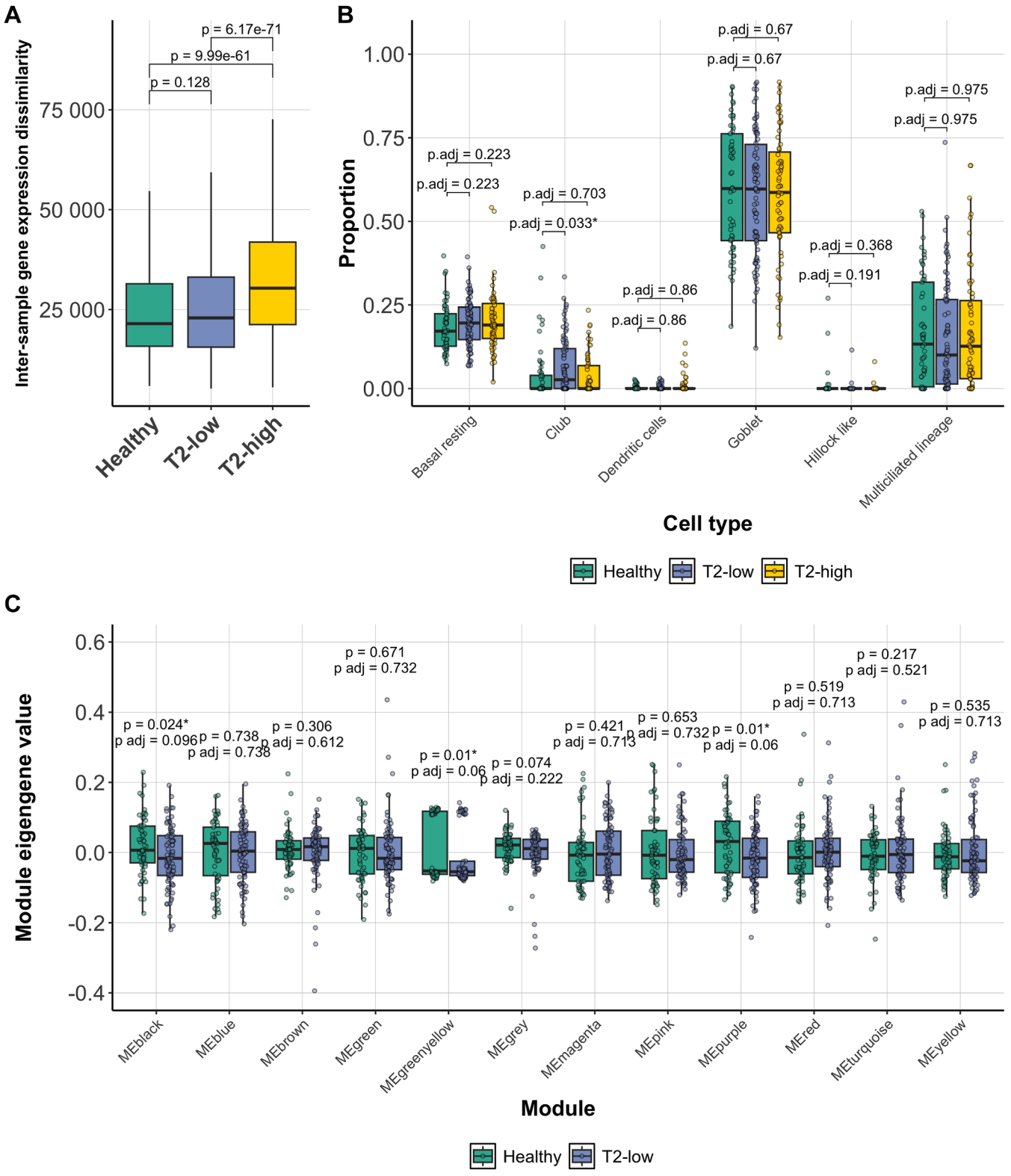


FIG 2. **(A)** Intersample gene expression dissimilarity within T2-low asthma, T2-high asthma, and healthy participants. Gene expression dissimilarity is expressed as pairwise Euclidean distances based on normalized expression values (CPMs) from all genes with measurable expression. Comparison was performed by Mann-Whitney *U* test. **(B)** Comparison of cell proportions between patients with T2-high and T2-low asthma and nonasthmatic controls as a reference group. Comparison was performed by Mann-Whitney *U* test. FDR-adjusted *P* values are shown. **(C)** Comparison of 12 coexpression gene modules identified by WGCNA between healthy controls and T2-low asthma subjects. Comparison was performed by *t* test; *P* values were adjusted for multiple testing by FDR.

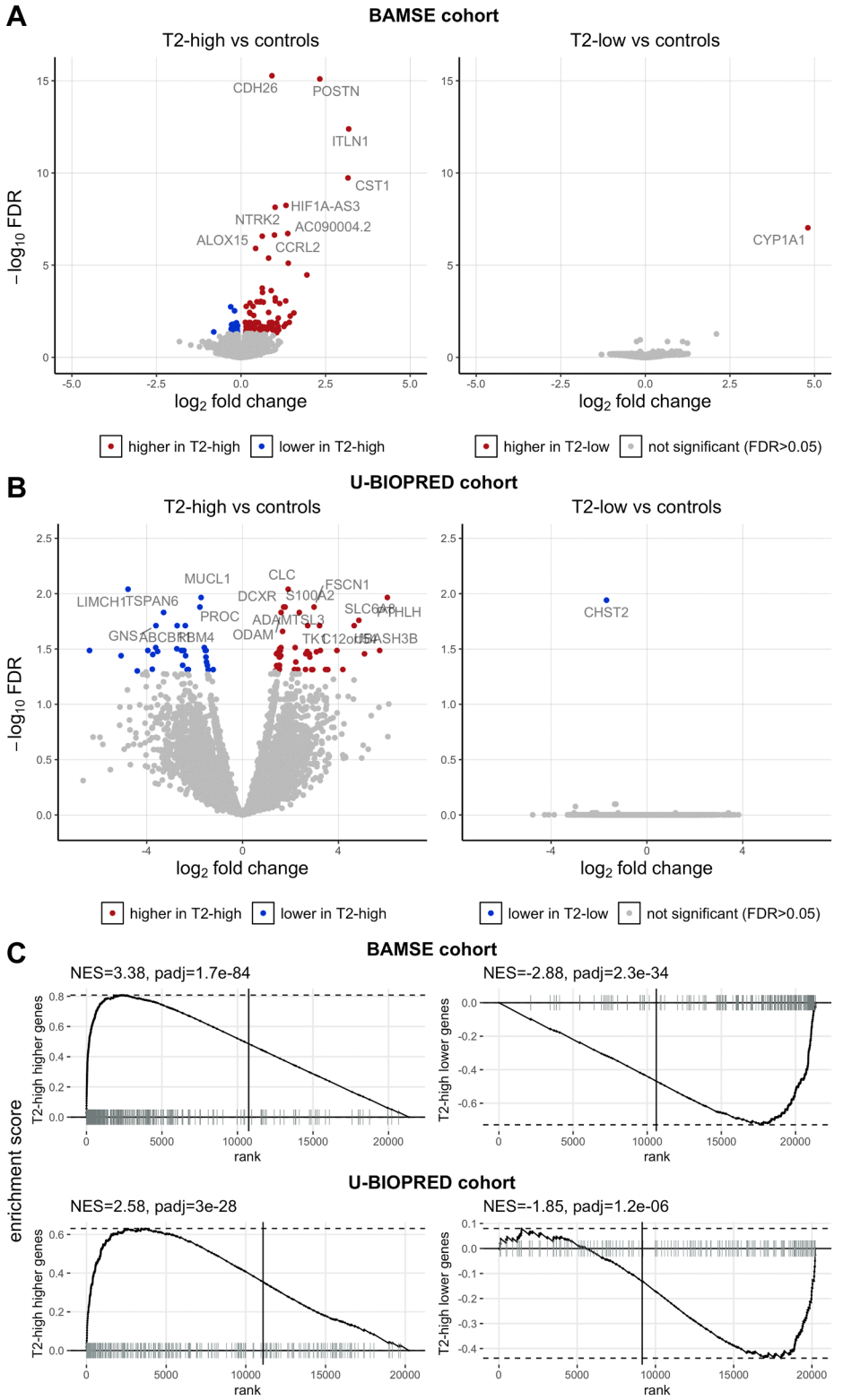


FIG 3. (**A** and **B**) Volcano plot representation of nasal brush DGE analysis of T2-high vs controls and T2-low vs controls in BAMSE *(A)* and U-BIOPRED *(B)* cohorts. Significance level was defined as FDR < .05. Model was corrected for age, sex, and smoking status. **(C)** Analysis of identified T2-high genes in ATLANTIS cohort as 2 gene sets (higher expressed and lower expressed) and genes ranked by $(-\log_{10}P\text{value})*\pm 1$ (depending on direction of change) from differential expression analysis comparing T2-high asthma vs controls in BAMSE and U-BIOPRED cohorts as background. *Vertical line* separates higher- and lower-expressed genes.

individual gene level. However, by taking gene coexpression into account, we identified a gene module associated with T2-low asthma characterized by genes involved in T-cell regulation and MHC.

In accordance with previous studies, we identified a T2-high gene signature, with the *CLCA1, POSTN,* and *CST1* genes being among the most highly expressed.[28-30] Previously we found similar genes to be highly expressed in the total group of patients with asthma compared to nonasthmatic controls.[10] These findings were likely driven by the patients with T2-high asthma because we did not identify any significant differences on an individual gene expression level. An observation that might be of interest was the identification of a gene module associated with T2-low asthma containing genes related to T-cell activation and MHC, with *HLA-DPB1* and *HLA-DPA1* being the top genes. Additionally, we found expression of genes from the "black" module to associate strongly to the predicted proportion of dendritic cells. The potential role of dendritic cells in the context of T2-low inflammation is of interest. Dendritic cells can prime T cells toward T_H1 or T_H17 responses rather than T_H2.[31] In addition, they can produce IL-12–stimulating T cells to produce IFN-γ, subsequently inducing macrophage activation.[32] Genes from the HLA-DP locus influence peptide presentation by MHC class II molecules, which are critical for $CD4^+$ T-cell activation. Genome-wide association study in an Asian population has identified single nucleotide polymorphisms in *HLA-DPB1*– and *HLA-DPA1*–associated asthma.[33] The role of these genes in different population and in relation to T2-low asthma should be further investigated.

In an attempt to assess molecular heterogeneity within each asthma phenotype, we conducted several analyses that revealed that T2-high asthma exhibits a higher degree of molecular heterogeneity than T2-low asthma in nasal transcriptome. This was an unexpected finding; Zounemat Kermani et al previously suggested T2-low asthma to be the more heterogeneous phenotype, having identified more clusters within T2-low asthma compared to the T2-high phenotype.[34] In this respect, it is important to note that their study used not only transcriptomic but also proteomic data, and it also assessed different compartments: sputum and blood. Notably, our findings show that T2-high asthma also exhibits molecular heterogeneity at the nasal transcriptome level. This heterogeneity within the T2-high asthma is supported by clinical observations, such as variable responses to inhaled corticosteroids (ICS) and biologics targeting T2 inflammation. Our findings highlight the need to further investigate potential subphenotypes within T2-high asthma to improve targeted treatment approaches.

There are several possible explanations for the fact that we did not find a robust signature of individual genes in T2-low asthma. First, T2-low asthma might be variable over time. Although we attempted to address this problem by selecting patients with stable T2-low asthma, no significant differences in gene expression were observed. Second, our study might be underpowered to identify smaller but still clinically relevant effects. Next, the underlying pathobiology of T2-low asthma might not manifest at the gene expression level but rather at the protein or epigenetic level. Therefore, future research focusing on other molecular layers could reveal mechanisms driving T2-low asthma. In addition, it is possible that in T2-low asthma the mucosal cells of the upper airways do not accurately reflect the disease biology in the lower airways, suggesting that lower airways or even extrathoracic mechanisms should be explored. This is supported by studies on the IL-6–driven lower airway gene expression profile, which distinguished asthma from controls in the lower, but not upper, airway.[35] Finally, it could be hypothesized that T2-low asthma is not associated with airway epithelial changes at all but rather involves mechanisms such as chronic activation of immune cells and their interaction with lower airway-resident cells, such as smooth muscle cells and fibroblasts.

Another interesting observation came from the cell-type deconvolution analysis. We observed an increase in the predicted proportion of club cells in T2-low asthma in ATLANTIS. Club cells act as progenitor cells for the airway epithelium and have the ability to differentiate into ciliated and goblet cells, but also to dedifferentiate into basal cells.[36,37] They are essential for maintaining airway homeostasis, detoxifying inhaled substances, and facilitating epithelial repair.[38,39] Interestingly, several studies showed messenger RNA and protein products of the gene *SCGB1A1,* also called *CC10* or *CC16* (club cell secretory protein 10/16) to be negatively correlated with sputum eosinophils, blood eosinophils, and FENO and was lower in T2-high asthma compared to T2-low and healthy participants.[40,41]

One of the challenges in defining T2-low asthma is the possible confounding effect of ICS on blood eosinophil counts and FENO levels. In this context, it is important to mention that the groups were balanced regarding ICS receipt and dosage. In addition, we attempted to address this limitation by analyzing our data with a stringent definition of T2-low asthma at all 3 study visits. Although the analysis of stable T2-low asthma yielded similar results, we acknowledge that this is a limitation. Another limitation of our study is that the presence of (allergic) rhinitis and the receipt of nasal steroid therapy was not systematically recorded in the ATLANTIS study, and we therefore could not account for this potential confounder in our analyses. Importantly, the prevalence of allergic sensitization (Phadiatop) was not significantly different between those with T2-low and T2-high asthma (72.1% vs 83.3%, $P = .210$). These findings suggest that allergic factors do not account for the observed nasal gene expression differences in T2-high and T2-low asthma compared to healthy controls.

In summary, in the current study, we explored nasal gene expression of T2-low asthma and did not find any significant genome-wide gene expression differences compared to healthy controls, likely a result of heterogeneity within the T2-low phenotype. We did observe higher club cell proportions and suggestive evidence of T-cell–mediated immune responses possibly contributing to T2-low disease pathogenesis. Future studies should assess the heterogeneity of T2-low asthma and consider lower airway samples in combination with other omics levels for finding common molecular markers of the disease.

DISCLOSURE STATEMENT

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RNA sequencing data have been submitted to the ArrayExpress (www.ebi.ac.uk/biostudies/arrayexpress) database under accession no. E-MTAB-16498.

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Key messages

- **Both T2-high and T2-low asthma are heterogeneous diseases.**
- **The nasal epithelium is a potential tool to diagnose and monitor disease activity in T2-high asthma.**

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